FEASIBILITY STUDY FOR THE MANUFACTURE OF ZERO GRAVITY PHARMACEUTICALS, IMMUNOLOGICAL, AND VIRAL AGENTS

FINAL REPORT

National Aeronautics and Space Administration
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by

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APPENDICES

(See Following Appropriate Section)

I. SUMMARY AND RECOMMENDATIONS

This study on the feasibility of manufacturing, purifying, isolating, or separating biochemical and cellular materials in space has lead to the following results:

1. There is little, if any, utility in processing pharmaceuticals in space due to their generally relatively small molecular size.

2. There is little confidence on the part of the industrial and scientific community in this type of approach for any application.

3. Nevertheless, there is a potential for the processing of cellular materials under zero gravity conditions. The only specific process suggested to us during the course of the study was for the production of vaccines from cellular material (Dr. Glick, President, Associated Biomedical Systems). The same respondent also suggested experiments on cell immortality in this context.

4. In general we attribute the negative and, at best, apathetic response received to the fact that the professionals involved take for granted that space processing as well as experiments are not cost effective. In addition, they probably have an understandable preference for having presently limited government funding directed to fields of direct interest to them. Thus, to arouse their interest some plausible proof of potential cost effectiveness would have to be clearly demonstrated to them.

5. In view of this situation, we resorted to indirect techniques for generating space process concepts, such as Medlar searches, internal discussions, interviews, and communications with scientists having even marginal interest, etc.

6. Based on this, we generated a list of "Potential Biochemical and Cellular Processes and Experiments" (Section VI). Some of these had to be disqualified, but others may lead to viable processes after further examination (see recommendations below).

7. In view of the above it is not possible at this time to come to definitive conclusions as to the practicability of space processing of socio-economically useful products in the classes examined.

8. It appears, however, that the most promising areas for further investigation are:

   - Processes which are based on the use of cellular materials which though the cells may not survive, may produce useful products.

   - Experiments with cellular materials to provide information vital to "on earth" research and processes.

In order to best utilize the above findings and to resolve the remaining questions as to the practicability of space processing for the class of materials under study we recommend that:

A. NASA enter into direct discussions with Associated Biomedic Systems as to evaluation and possible implementation of suggestions under 3 above.

B. When the T and B lymphocyte separation experiments in space and/or the evaluation under A. above are successfully completed, scientists in research and industrial organizations should be re-approached for discussion of suggestions contained in the list of Section VI, as we believe such a demonstration would create a better climate for unbiased discussions.

II. INTRODUCTION: METHODS AND RESULTS

A. PURPOSE OF THE STUDY

The National Aeronautics and Space Administration requested Arthur D. Little, Inc., to study the feasbility of extracting, isolating, purifying, separating, or preparing medical and biological products of high socio-economic value in space. In particular, the study was designed to concentrate on the isolation or purification of virals, pharmaceutical and immunological agents by means of electrophoresis, as the Apollo 16 flight demonstrated that a mixed population of latex spheres (.2 and .8 microns) could be successfully separated by electrophoresis in space and without sedimentation with minimal convection. This prompted NASA scientists to look into the possibility of carrying out types of biochemical experiments that would utilize space for medical purposes.

Our program did not deal, however, with the general biological experiments that can be performed in space, but with the very specific problems of producing useful materials in sufficient quantities for any given biomedical application, and, if possible, of finding a potential manufacturer for such materials.

Benefits to humanity must be established on the basis of social and economic values and the competitive nature of the process when compared with equivalent technology on earth. Furthermore, it implies, that if a material is found to cure a given disease, this disease should have socio-economic values beyond emotional considerations to justify performance of the given material isolation.

B. METHODS

This program posed the very challenging problem of designing an approach which was neither known nor predictable at the outset. Thus, not only did we not know what substances we were looking for among pharmaceutical, viral and immunological agents, but, in addition, it soon became evident that space conditions were not per se competitive with existing earth production procedures and, furthermore, there was little interest from the potential commercial users of these techniques.

Accordingly, we decided to take a multidimensional approach which would give us some idea on how to identify possible materials, techniques and users. Three main lines of approach were undertaken in parallel, as discussed in the following.

1. Definition of Biophysical and Biochemical Properties That Are Different in Space

The main object was to limit the set of possible materials that one could possibly look at. We asked questions related to sedimentation times, charge to mass ratios, etc., of various molecular and cellular entities as well as the effect of gravity on various biophysical techniques. As large gravitational fields are generally introduced on purpose in biochemical and biophysical separations (such as ultracentrifuge work), relevant problems required extensive thought and consultation. Our conclusion was that only large biological particles, such as cells, or functional cellular groups or tissues had potential isolation or purification problems for which space could, at least in principle, provide an answer. Smaller particles, including subcellular material, take extremely long times to settle and thus offer little or no potential. Furthermore, organized cellular structures can be separated on earth by microsurgical dissection techniques, now well automated, while most cells of interest cannot be kept viable for the long periods of time considered for the program (including preshipment wait, flight time, return to appropriate laboratories, etc.). In addition, freezing before return to earth for storage would tend to destroy important functional properties of most cells.*

This precludes in most instances the use of space processed viable cells per se and thus we believe limits us to possible exploitation of products produced by and from cells under space conditions and which do not lose their activity by freezing or other return shipment requirements. In addition, in experimental usage, return of information may be all that is needed.

In terms of the biophysical techniques that are affected by gravity, the other input to be considered, electrophoresis, was one of the two found to have any relevance, as zero gravity eliminates problems of convection and the requirement for a supporting material.

Another possible technique, more on the purely biological side, has to do with cells that cannot grow when attached to glass and might grow or reproduce indefinitely in space, just as cancerous cells which do not exhibit contact inhibition.

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*Acceleration and deceleration should not affect cell viability as cells survive normally accelerations in extremely large gravitational fields in the ultracentrifuge for separation into various fractions.

Our conclusions for this part of the study were:

- Only two techniques would show potential differences in space: electrophoresis and cell culture in suspension.
- Only experiments with cells can be done.
- Only small molecules extracted from or information about the cells themselves is worth returning, in the general case.

Some of the appropriate possible experiments are discussed in the section on potential zero gravity processes and experiments. (Section VI)

During performance of this task we also studied competitive technology: this includes an extremely fast cell sorting device made by Los Alamos Scientific Laboratory and a dynamic gradient cell separator which, when used in conjunction with a Ficoll gradient can separate T and B lymphocytes on earth.

2. Users Requirements

A second technique consisted in approaching a significant sample of pharmaceutical companies which manufacture macromoleculars, cellular biochemicals, vaccines and immunologicals. These companies were chosen by selecting those which manufacture macromoleculars listed in the Physicians Desk Reference Book, and which, presumably, should have social and economic value.

We found out, however, that most of these macromoleculars have no well-determined use, many are placebos or digestive enzymes (Table 5). In addition, as Table 6 shows, most of the bulk of pharmaceutical sales come from tranquilizers, contraceptives, etc., and have little to do with the types of substances that can be separated to advantage in space. This indicates an immediate economic limitation in terms of the inertia to be overcome by the pharmaceutical companies. While many responses were obtained in response to our letters (Appendix 2) and our telephone calls, no positive output was obtained from the companies, with the exception of MERCK SHARP AND DOHME which indicated that it is working on a system to simulate zero gravity on earth by utilizing centrifugation and diffusion across an equilibrium boundary. An additional important output was obtained as a by-product from discussing this project with relevant personnel at the companies, namely that little or no electrophoretic equipment is used in production. Some is used, however, in research at the pharmaceutical companies. This was puzzling as we had already found out as a result of our literature searches that seven of the 70 most cited journal articles for the period 1961-1972 were on the subject of electrophoresis methodology (Table 8) and that the electrophoresis equipment market is of the order of $22 million. Telephone contacts with electrophoresis manufacturers confirmed that very little of their equipment is sold to pharmaceutical companies; most equipment goes to

academically-oriented research laboratories.

For this reason, we decided to contact research laboratories specifically interested in electrophoresis and, in particular, cell electrophoresis. The first step in the process was the literature search described below, which served to generate specific research ideas and helped to identify fields of work of various scientists. The response was good, but the ideas submitted poor, nebulous or only expressing a willingness to collaborate "if something happened." (See Appendix 4) Another approach consisted in informally talking to various scientists at several research and contractors conferences which included a meeting on mammalian cell surface at Los Alamos Scientific Laboratory and a National Cancer Institute contractors conference. The interest was marginal on the part of most investigators, except for those which found the problem academically intriguing.

3. MEDLAR Search

We requested the National Library of Medicine to perform a computer search of the articles in various areas of techniques, macromolecular separations, cellular electrophoresis, etc., for the years 1971-1973. Organized references from this study are given in Appendix 3 . We selected specific articles for introductory discussion and looked in detail at those which seem to warrant further attention. We then contacted the scientists who had written the given article, as described above in 2. The various substances and techniques we looked into are given alphabetically in Appendix 4 .

4. Internal Generation of Ideas

Because of the poor external response to the NASA program, we decided to generate internal ideas at Arthur D. Little, Inc., and are providing those in Section V. Again, we exposed many scientists to these and their usual reaction was a "wait and see" response; they would like to see the experiments performed, but nobody wants to endorse them directly. The importance attached to these experiments is, at present, hard to assess; multiple layers of screening and evaluation will certainly have to precede experiments of the order of cost envisaged and, before this is done, it seems practically impossible to obtain relevant, unbiased judgment from peer scientists. Part of the problem may be naturally solved once the experiments proposed on T and B lymphocytes, lipoproteins, etc., are run and the scientific community can judge directly the merits of this type of space experimentation.

5. Clinical Tests

We have also analysed the area of common clinical tests (see Appendix 6) to determine what types of improvement are required. Suggestions for space tests have been included with the research suggestions.

III. BIOPHYSICAL CHARACTERISTICS REQUIRED OF CANDIDATE COMPOUNDS AND TECHNIQUES

A. INTRODUCTION

In conjunction with the active search for appropriate candidate materials that could have potential use to humanity--performed by contacting various pharmaceutical companies, instrument manufacturers, scientists, etc.--we carried out an analysis of the characteristics that any candidate compound should have in order to be advantageously separated and purified as compared to other materials in space.

The following questions had to be answered in order (see Flow Chart 1, for appropriate outputs):

- What are the physical characteristics of space that makes it different from the earth laboratory from the biological, biochemical, biophysical or physical points of view, i.e., what does a molecule, cell, virus, etc., "see" in space that is different from earth?
- Given the above, which are the substances, biological materials, etc., that could be considered potential candidates?
- What techniques are used on earth to isolate the potential candidates?
- How does space affect the isolation, purification, or fractionation technique itself?
- Can the candidates be put in orbit and brought back to earth without deterioration of the material?
- Is the experiment actually representing a unique zero g situation or can the same factors be reproduced on earth?

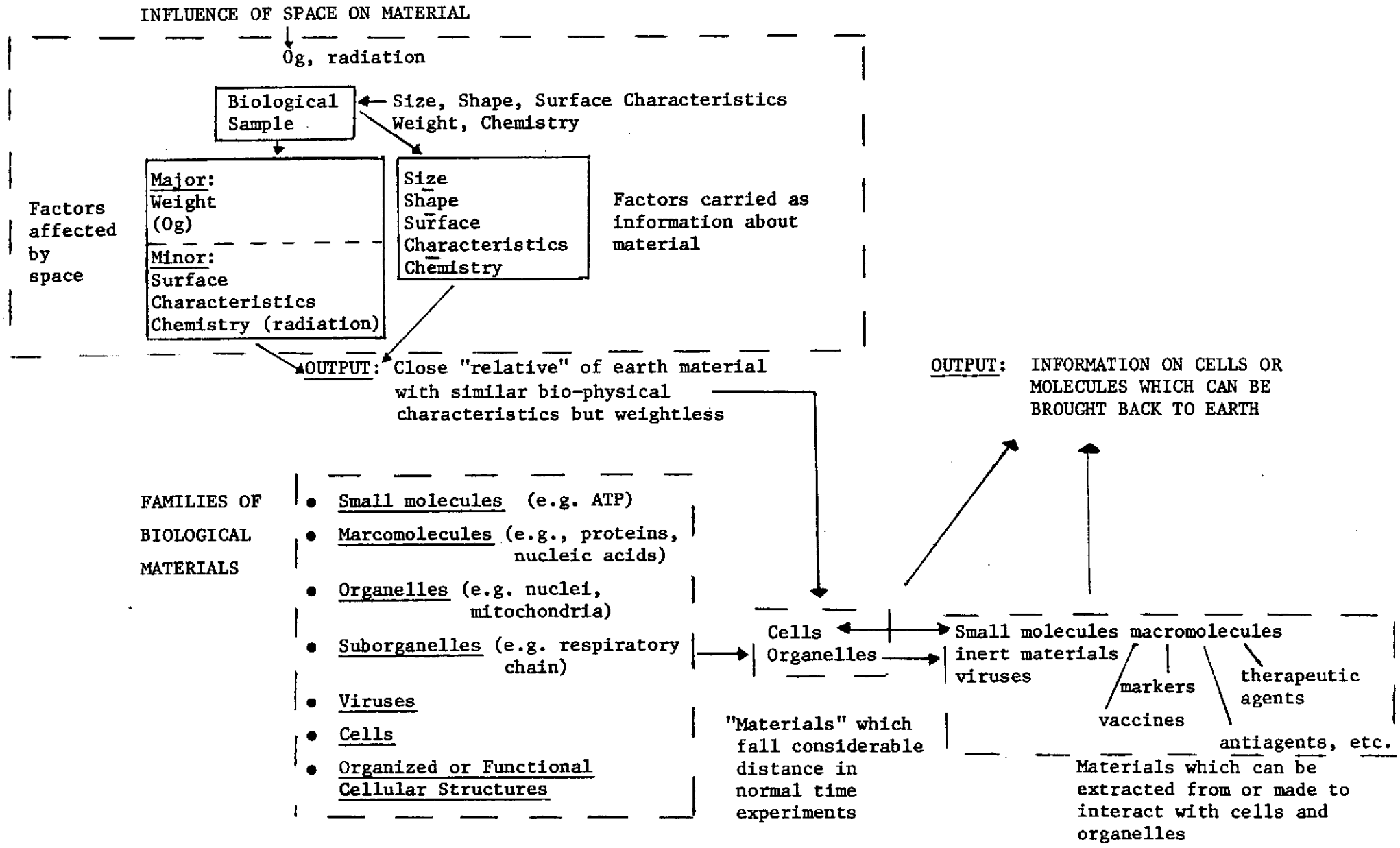
Some of these questions are described below, not in reference to specific materials,but in terms of the general requirements of the experiment.

B. SPECIFIC FACTORS

1. Physical Characteristics of Biological Entities

Any molecule, virus or cell has associated with it a size, shape, weight, and certain surface characteristics such as attached charges, special receptors and reactive groups. In addition the structure should

FLOW CHART 1


INFLUENCE OF SPACE ON MATERIAL
0g, radiation
Biological Sample
Size, Shape, Surface Characteristics
Weight, Chemistry
Factors affected by space
Major:
Weight
(0g)
Minor:
Surface
Characteristics
Chemistry (radiation)
Size
Shape
Surface
Characteristics
Chemistry
Factors carried as information about material
OUTPUT: Close "relative" of earth material with similar bio-physical characteristics but weightless
OUTPUT: INFORMATION ON CELLS OR MOLECULES WHICH CAN BE BROUGHT BACK TO EARTH
FAMILIES OF BIOLOGICAL MATERIALS
• Small molecules (e.g. ATP)
• Marcomolecules (e.g., proteins, nucleic acids)
• Organelles (e.g. nuclei, mitochondria)
• Suborganelles (e.g. respiratory chain)
• Viruses
• Cells
• Organized or Functional Cellular Structures
Cells
Organelles
Small molecules macromolecules
inert materials
viruses
markers
therapeutic agents
vaccines
antiagents, etc.
"Materials" which fall considerable distance in normal time experiments
Materials which can be extracted from or made to interact with cells and organelles

be considered as an open system which can interact with the environment through exchange of energy and in the case of cells, through exchange of small molecules which move actively or passively through the cell membrane. The only relevant effects of space are: (a) the lack of weight in space, (b) the biochemical effects of high radiation levels when unshielded (such as mutations, etc.) and (c) the possibility of cell growth not limited by contact inhibition with other cells or container vessels.

2. Effects of Space on Biophysical Techniques for the Study of Molecules, Cells and Viruses

a. Techniques to study size: Chromatography and membrane transport processes are not affected by gravity.

b. Techniques to detect shape: This may involve microscopes as detecting devices (either manually operated or automatic) X-rays and electron sources. No important effects of gravity are present.

c. Density Techniques: Equilibrium or dynamic sedimentations ranging from 1g to ultracentrifuge fields clearly depend on gravity for achieving sedimentation, separation, etc.

Sedimentation, centrifugation and ultracentrifugation all depend on the relative velocity and equilibrium distribution of materials of different density under the action of a potential energy gradient; particles are separated from solution forcing them into separate compartments of the fluid under the influence of this gradient.

In a gravitational field particles in a fluid settle at a velocity given by Stokes' Law:

$$V = \frac{2}{9} \frac{r^2 g (\rho - \rho o)}{\eta} \qquad (1)$$

Where: V is the terminal settling velocity
ρ is the density of the particle
ρo is the density of the solvent
η is the viscosity of the solvent
r is the particle radius, and
g is the strength of the gravitational field.

It is evident from Equation (1) that large particles settle rather rapidly under a gravitational field, while small particles settle extremely slowly. (A 0.1 micron particle of density 1.1 settles in water under earth gravity at only 8×10^{-4} cm/hr.) Red cells in blood settle only a few centimeters per hour. Thus, normal sedimentation is effective only for the separation of fairly large particles.

Centrifugation provides a means of increasing the apparent gravitational field by as much as 25,000 times. Thus, small particles such as blood cells can be efficiently separated within reasonable times. Ultracentrifugation extends the ordinary centrifugation process by operating at much higher speeds. This allows separation of very small particles (e.g., viruses) and even allows separation by fractionation of large dissolved molecules (such as blood proteins). Suspending solutions of varying fluid densities are frequently used to maintain the molecular solutions in discrete layers (i.e., to prevent remixing of the separated molecules). Sucrose solutions are frequently used to produce these solution gradients.

Natural sedimentation is impossible in a zero gravity environment, since it depends on the force of gravity to produce the action. Centrifugation and ultracentrifugation would be relatively unaffected by a zero gravity environment since the induced acceleration forces are large compared to the force of earth gravity.

3. Effects of Gravity on Techniques for Measuring Surface Properties

Clearly, techniques based on specific attachment or adsorption are not affected by gravity. Electrophoresis, one of the most important techniques for surface property measurements, is affected by gravity in two ways when fluid electrophoresis is performed without a supporting medium: (a) large particles sediment as they move in the electrophoresis tube, and (b) the boundaries of the vessel and center have different temperatures because of Joule heating; as the hotter part of the solution is lighter and the colder part sinks, convection and mixing results. This can be reduced on earth by working at a temperature close to that of maximum solvent density (4°C for aqueous solutions). The change of liquid density with temperature is small or negligible at that temperature and convection minimal. (In the case of electrophoresis on a supporting medium, however, the heat effects change the net particle mobility but there is no convection.) Convection effects can be minimized by utilizing narrow electrophoresis tubes. The lack of convection and sedimentation in space electrophoresis was clearly demonstrated by the separation of two populations of latex spheres which had been mixed, although there were electrostatic effects which prevented complete particle separation.

4. Materials and Techniques of Choice

The most relevant situation in space consists of running experiments in a fluid electrophoresis cell of a biological particle that would normally sediment on earth during the times of the experiment. Typical biological units are listed on Table 1 and sedimentation rates on Table 2. Clearly, for taking into account the amount of time needed for any of the units considered in a typical purification or isolation experiment, only cellular space separation and tissues or organized cellular structures are worth considering.

TABLE 1

ISOLATION OF CELLULAR ELEMENTS

BIOLOGICAL UNIT	SEPARATION TECHNIQUE	PRESENT PROBLEMS
• Marcomolecules (Proteins, D.N. A., R. N. A.)	Centrifugation Chromatography	Separation of acidic nuclear proteins
• Organelles - Nuclei - Mitochondria - Lysosomes (De Duve, 1963) - Glycogen granules (Greene, Hirsand, Palade, 1963) - Histamine granules (Hagan, Barrnett and Lee, 1959) - Catecholamine granules (Blascko, 1959) - Vasopressin granules - Oxytocin granules (Weinstein, Malamed, Sachs, 1961; Barer et. al., 1963) - insulin granules (Lindall et. al., 1963)	Centrifugation	Isolation of mitotic spindles (microtubules) granules containing specific enzymes or transmitters
• Viruses	Chromatography Electrophoresis	See purification required, Appendix 7
• Suborganelles - Nucleoli (Maggio et. al., 1963) - Electron transport chain fragments (Lusena and Dass, 1963)		
• Whole Single Cells	Sedimentation Centrifugation	See text
• Organized Cellular Structures (tissues) - Renal Glomemlae (Cook; Pickering, 1969) - Separation of sperm heads and tails (Masaki & Hartree, 1962)	Disection, etc.	

TABLE 2

SETTLING RATE OF PARTICLES

Particle	Density gm/cc	Molecular Weight	Mean Radius Å	Sed Coeff cm/sec dyne	Settling Rate in Water in 1G Field cm/sec	Distance Settled in 1 Hour
Ribonuclease	1.37	12,400	16.8	1.6×10^{-13}	1.6×10^{-10}	5.6×10^{-7}
Serum Albumin	1.36	66,000	29.5	4.31×10^{-13}	4.2×10^{-10}	1.5×10^{-6}
Tobacco mosaic virus	1.37	50,000,000	268	170×10^{-13}	1.7×10^{-8}	6.0×10^{-5}
Human lymphocytes	1.072		10μ		2×10^{-4}	about 1 cm

Cell surface groups that contribute to variations in charge mobility are studied indirectly by treating the cell surface with enzymes which specifically remove groups from the surface. Common groups which contribute to the electrokinetic charge are (see J.N. Mehrishi and A.E.R. Thomson, (1968) Nature 219, 1080.) given in Table 3.

TABLE 3 SOME OF THE GROUPS WHICH CONTRIBUTE TO CELL CHARGE

GROUP	SUSCEPTIBILITY TO (enzyme)	CELLS	REFERENCE
α-carboxyl of NANA (N-acetyl-neuraminic acid)	Neuraminidase	Erythrocytes	Piper, 1957 Cook, et al, 1961 Eylar et al, 1962
		Blood Platelets Lymphocytes	Madoff, et al, Mehrishi, 1968
		Leukocytes	Vassar, et al, 1969
Phosphate Groups	Ribonuclease	Lymphocytes Tumor Cells	Mayhew and Weiss, 1968 Mayhew and Weiss, 1968
Phosphate Groups	Alkaline Phosphatase	Blood Platelets	Mehrishi, 1970b

Small molecules, macromolecules, viruses, organelles and suborganelles fall too slowly under the action of gravity.

Organized cellular structures, however, may be separated on earth by microdissection. Cells, on the other hand, will be extremely hard to keep viable after all transfers and waiting periods required for space shipment. Furthermore, functional properties in most cases will not survive freezing. Critical cell types, such as bone marrow, cannot survive for more than a few days, even if strict environmental conditions are met.

This leaves two options in terms of material return:

- small molecules produced by the cells, or
- information on experiments performed on cells (this could

include observations performed aboard as well as bringing back the nonfunctional dead cell to earth).

A third possibility, which was not originally required from our program, but may be useful, consists in running model systems (such as quartz spheres containing mosaics of charged groups similar to the charge groups present on cell surfaces).

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IV. APPROACHES BASED ON USERS AND NEEDS

This task had the following objectives: (a) obtain suggested experiments and candidate materials from the pharmaceutical, research, and clinical groups, and (b) utilize these groups as a feedback system to check our own selected ideas.

In order to contact appropriate pharmaceutical companies on some rational basis, we assembled the list of macromolecules shown in Table 4, and contacted manufacturers of these. This list comprises all the macromolecules with known therapeutic value and are included in the Physicians Desk Reference. At that time, we had not decided to concentrate on cellular material and we hoped to find a pharmaceutical process with potential economic and social value which could take advantage of space extraction or purification. The exercise was instructive in itself. First, we found out that most of these materials do not have a great social value, as shown in Table 5 which describes the present sources and applications of these materials. Second, these materials have little economic value and do not comprise the bulk or even an important part of the pharmaceutical industry dollar output. (See Table 6 for types of products with high sales.)

The manufacturers were contacted by letter and by telephone. In those cases where letters were not answered, a follow up was required. Responses were generally negative as shown in Summary Table 7. Response letters are presented in Appendix 2.

A very surprising finding of this part of the study was that there seemed to be no use of electrophoresis equipment in pharmaceutical production processes. We checked this fact with some of the leading manufacturers of electrophoresis equipment to find out who buys this type of instrumentation (the market is estimated to be $22 million) and found out that most equipment is sold to research scientists.

While, as stated, electrophoresis is hardly used in industrial processes, the biological research area is dramatically more fertile. Electrophoresis is an important and commonly used tool in contemporary basic research. A recent study by the Institute for Scientific Information (Garfield, E., Current Contents, January 9, 1974, and Garfield, E., Current Contents, February 6, 1974) reveals that seven of the 70 most-cited journal articles for the period 1961-1972 are on the subject of electrophoresis methodology. These articles are listed in Table 8.

TABLE 4

LIST OF MACROMOLECULES WITH KNOWN THERAPEUTIC VALUE WHICH ARE INCLUDED IN THE PHYSICIANS DESK REFERENCE

Allergens
Antigens
Anti-Serum
Immunosuppresive
Plasma Fractions
Serum
Toxoids
Adrenocorticotropic Hormone
Albumin, Normal Serum
Alginic Acid
Amylolytic Enzyme
Anterior Pituitary Hormones
Bromelains
Cellulase
Chondroitin Sulfate
Chorionic Gonadotropin
Chymotrypsin
Collagenase
Desoxyribonuclease
Enzymes (Debridement)
Enzymes (Digestant)
Enzymes (Fibrinolytic)
Fibrinogen (Human), U.S.P.
Glucagon for Injection U.S.P.
Heparin Preparations
Insulin
Intrinsic Factor Concentrate
Lipolytic Enzyme
Papain
Pepsin
Secretin
Sodium Nucleate
Streptokinase-Streptodornase
Thyroglobulin
Trypsin

TABLE 5

USES OF REPRESENTATIVE MACROMOLECULARS LISTED IN THE PHYSICIAN'S DESK REFERENCE

Name	Use	Main Chemical Components	Source
• Adenocorticotropic Hormone Acthar (Armour)	Rheumatoid disorders Collagen diseases Dermatologic diseases Allergic states Ophthalmic diseases Respiratory diseases Hematologic disorders Neoplastic diseases Edematous state	Corticotropin U.S.P. Gelatin Phenol	Anterior petuitary hormone
• Albumen, normal serum Albuspan (Park Davis) Albuminar (Armour) Alburnin (Cutter)	Shock Hypoproteinemia Hyperbelerubinemia and erythroblastosis fetalis	Serum albumin isotonic iso-osmotic with pooled whole human plasma	Venous and/or placental plasma
• Alginis Acid Marion Pepsin Lipolytic	Temporary relief of heartburn caused by reflux of contents from stomach	Alginic acid 200 mg Aluminum hydroide Sued gel 80 mg Sodium bicrabonate 70 mg	When chewed produce a viscous demulcent antiacid foam which coats
• Amylolytic enzyme Medical products paramericana Hoechst Geriatric Warren-Teed Kremers-Urban Ulmer Rowell	Flatulence, nervous indigestion, gastric idstress, intestinal hypermotility and spastic colon	Lipase Amylase Protease Bile salts	

TABLE 5 (Continued)

Name	Use	Main Chemical Components	Source
• Anterior Pituitary Hormones Armour	Rheumatic disorders Collagen diseases Dermatologic diseases Allergic states Ophthalmic diseases Respiratory diseases Hematologic disorders Neoplastic diseases Edematous state	Corticotropin	Anterior pituitary hormone
• Bromelains Rorer	Supplement and augment standard therapeutic procedures for reduction of inflammation and edema to ease pain, speed healing and accelerate tissue repair	Concentrate of proteolytic enzymes	Pineapple plant
• Cellulase Wampole Dorsey Ulmer Hoechst Geriatric Kremers-Urban	Reduces flatulence, relieving constipation, improving appetite or digestion from eating vegetables with high cellulose content--cucumbers, lettuce, etc.	Cellulase, amylase, protease, lipase	Aspergillus niger (cellulase)

TABLE 5 (Continued -3-)

Name	Use	Main Chemical Components	Source
• Chondroitin sulfate Marcen	Prevention and treatment of atherosclerosis Useful adjuncts - Angina pectoris - Peripheral vascular diseases - Intermittent claudication - Reduces blood cholesterol and lipoprotein levels	Pancreas extract (epinephrine--neutralizing Pancreas extract (lipotropic fraction)	Acid-alcohol treated pancreas 80% alcohol extractive of deproteinated pancreas
• Chorionic gonadotropin Ayerst Research supplies	Given in an attempt to stimulate the interstitial cells of the testes to produce androgen - Delayed adolescence - Hypogonadotropic eunuchoidism	Human chorionic gonadotropin (APL)	
• Chymotrypsin Armour Wampole Warren-Teed Merrell-National	Indicated for enzymatic zonulysis for intracapsular lens extraction. Dissolves zonular fiber attached to the lens by proteolytic enzymatic action. As anti-inflammatory action in cases of urologic surgery, episiotomy, surgery of head and neck.	Chymotrypsin	Proteolytic enzyme
• Collagenase Advance Biofactures Knoll	Debriding dermal ulcers and severely burned areas	Collagenase activity	Fermentation of clostridium histolyticum

TABLE 5 (Continued -4-)

Name	Use	Main Chemical Components	Source
• Desoxyribonoclease Parke, Davis	Debriding agent for inflammatory and inffected lesions: general surgical wounds ulcerative lesons-tropic decubitus, stasis, arteriosclerotic second and third degree burns circumcision and episiotomy irrigating agent in: infected wounds-abscesses fistulae, and sinus tracts otorhinolaryngologic wounds superficial hematomas	Fibrinolysin Desoxyribonuclease	From two lytic enzymes: Fibrinolysin: bovine enzyme Desoxyribonuclease: isolated in a purified form from bovine pancrease
• Enzymes (Debridement) Armour Parke, Davis Rystan Flint	Digest necrotic tissues pyogenic membranes and crusts to cleanse wounds and remove foul odors	Neomycin palmitate Trypsin-chymotrypsin	From mammalian pancreas glands

TABLE 5 (Continued -5-)

Name	Use	Main Chemical Components	Source
• Enzymes (Digestant) Lakeside Warren-Teed Kremers-Urban Nutrition Control VioBin Brunswick Standard Process	Disorders of motility of the lower gastrointestinal tract: irritable colon colitis, mild chronic ulcerative rectospasm acute enterocolitis diverticulitis	Mepenzolate bromide or phenobarbital	
• Enzymes (Proteolytic) Warner-Chilcott	Reduction of edema	Proteolytic enzyme	Caraca papaya
• Fibrinogen (human) U.S.P. Cutter	No information	No information	No information
• Glucagon for Injection U.S.P. Lilly	Counteracts severe hypoglycemic reactions in diabetic patients--or during insulin shock therapy in psychiatric patients	Straight chain polypeptide containing twenty-nine amino acid residues	Produced in the pancreas (alpha cells of the island of langerhans)
• Heparin Preparations Upjohn Abbott Riker	Inhibits the coagulation of blood and the formation of fibrin clots both *in vitro* and *in vivo*	Sodium heparin	Intestinal mucosa
• Insulin Squibb Burroughs Wellcome Lilly	Keeps sugar content at a balance		Antidiabetic principle of beef pancreas or pork pancreas

TABLE 5 (Continued -6-)

Name	Use	Main Chemical Components	Source
• Intrisic factor concentrate Breon Upjohn Lederle Lilly	Relief of bronchospasm in bronchial asthma	Ethylnorepinephrine	Synthetic sympathomimetic amine
• Papain Boyle Rystan	Antihistaminic Decongestant, analgesic for relief of nasal conjectant muscular aches and pains and common pollen allergies	Phenylephrine HCI Pheniramine maleate Salicylamide Phenacetin	
• Secretin Warren-Teed	Diagnosis of pancreatic disorders	Secretin-Boots	Hormone, porcine duodenal mucosa freeze dried, refined
• Sodium nucleate Marcen	When leukopenia occurs in certain pyogenic diseases normally accompanied by leukocytosis, Vero-Zyme may be used as an adjunct to suitable antibiotic therapy to promote leukocytoses and phagocytosis	Sodium nucleate	
• Streptokinase-streptodornase Lederle	Treatment of edema associated with infection and trauma	Streptokinase Streptodornase	Strain of streptoccus

TABLE 6

ESTIMATED MONTHLY CONSUMER SALES OF SELECTED ETHICAL PHARMACEUTICAL PRODUCTS[1]

(Mfrs. sales dollars)

Product	Manufacturer	Therapeutic Class	Estimated Domestic Sales (M$) 1973 June	July	August	September
Loestrin Group	Parke, Davis & Co. (Warner-Lambert Co.)	progestogen	25	40	50	75
Nor-Q.D.	Syntex Corp.	progestogen	40	40	50	40
Micronor	Ortho Pharmaceutical (Johnson & Johnson)	progestogen	30	30	25	20
Darvon-N Group	Eli Lilly & Co.	analgesic	900	1,050	1,000	900
Darvocet-N	Eli Lilly & Co.	analgesic	450	500	625	675
Dolene 65 Group	Lederle Laboratories (American Cyanamid Co.)	analgesic	20	30	25	25
SK-65 Group	Smith Kline & French Laboratories (SmithKline Corp.)	analgesic	90	80	90	75
Sinequan	Pfizer Laboratories (Pfizer, Inc.)	tranquilizer, antidepressant	1,300	1,800	1,800	1,900
Adapin	Pennwalt Pharmaceutical Products (Pennwalt Corp.)	tranquilizer	75	85	85	110
Tranxene	Abbott Laboratories	tranquilizer	450	650	700	700
Promapar	Parke, Davis & Co. (Warner-Lambert Co.)	tranquilizer	25	20	20	20
Chlor-PZ	USV Pharmaceutical Corp. (Revlon, Inc.)	tranquilizer	25	20	20	20
Tofranil PM[2]	Geigy Pharmaceuticals (Ciba-Geigy Corporation)	psychostimulant	–	–	–	20
Aarane	Syntex Corp.	bronchodilator	25	150	200	225
Intal	Fisons Corp.	bronchodilator	–	200	150	100
Alupent[2]	Boehringer Ingelheim Ltd.	bronchodilator	–	–	–	20
Sanorex	Sandoz Pharmaceuticals (Sandoz-Wander, Inc.)	antiobesity	–	170	375	325
Pondimin	A.H. Robins Co.	antiobesity	–	60	200	175
Voranil	USV Pharmaceutical Corp. (Revlon, Inc.)	antiobesity	–	90	125	100
Principen/Probenecid	E. R. Squibb & Sons (Squibb Corp.)	antibiotic	–	–	100	75
Polycillin PRB	Bristol Laboratories (Bristol-Myers Co.)	antibiotic	–	–	60	60
Septra	Burroughs Wellcome & Co. (Wellcome Foundation Ltd.)	antibacterial	–	–	125	400
Bactrim	Roche Laboratories (Hoffmann-La Roche, Inc.)	antibacterial	–	–	250	300

1. Monthly estimates are based on product sales to consumers and exclude sales for inventory purposes.
2. New product.

Source: Arthur D. Little, Inc.

TABLE 7

RESPONSE FROM MANUFACTURERS

COMPANY	COMMUNICATION *LETTER	COMMUNICATION VERBAL	DATE OF RESPONSE	SUMMARY OF RESPONSE
Abbott	+	-	11/1/73	Grant H. Barlow is working with Marshall Space Flight Center now on a possible experiment for the Soyez-Apollo Flight in 1975. Would like to discuss with us.
American Cyanamid, Lederle Labs	+	-	11/30/73	Mr. Ringler, Director of Research is unable to suggest any appropriate experimentation.
American Hospital Supply	+	-	-	-
Wyeth (Am. Home Products)	+	-	-	-
Armour Pharmacue-tical Co.	+	-	10/25/73	Armour has no interest at present but will send a copy to Professor Craig Wall at Baylor College of Medicine, Texas Medical Center.
Ayerst, Div. Am. Home Products	+	-	10/19/73	Copies of the letter forwarded to the administrators of the Research and Development Department.
Baxter	-	+	10/15/73	-
Becton-Dickinson	-	+	10/16/73	-
Brunswick Labs.	+	-	-	-
Cutter Labs.	+	-	-	-
Difco Labs., Inc.	+	-	-	-
Dome-Miles Lab., Inc.	+	-	-	-
Dorsey Labs.	+	-	-	-

*all letters were mailed on 10/17/73

TABLE 7 (continued)

COMPANY	COMMUNICATION LETTER	COMMUNICATION VERBAL	DATE OF RESPONSE	SUMMARY OF RESPONSE
Dow Pharmaceuticals	+	-	11/27/73	At the present time they do not have a project which would qualify for the program.
Flint-Div. of Travenol (Baxter)	+	-	-	-
Hoechst Pharmaceutical Co.	+	-	-	-
Hollister-Stier	+	-	11/15/73	Company sent a folio containing literature and information on different products manufactured by the company for the diagnosis and treatment of the allergic patient, also instruction sheets which accompany the various extracts. Interest in helping to devise a TINE system.
Johnson & Johnson Orthodiagnostic Div.	+	-	10/25/73	No contribution to the project.
Kremers-Urban	+	-	-	-
Eli Lilly Co.	+	-	-	-
Marion Labs.	+	-	10/24/73	Company is not involved in this area and most likely will not be in the immediate future.
Merck Sharp & Dohme	+	-	10/22/73 1/15/74	At present they are not able to suggest a substance which would be best isolated using electrophoresis under zero-gravity conditions. Two other areas of potential interest for an experiment is space: the rapid and profound demineralization, the examination of the circadian rhythm (space agency has done work in rat area).

*all letters were mailed on 10/17/73

TABLE 7 (continued)

COMPANY	COMMUNICATION *LETTER	COMMUNICATION VERBAL	DATE OF RESPONSE	SUMMARY OF RESPONSE
Organon, Diagnostics Div.	+	-	-	-
Pfizer Labs.	-	+	10/15/73	No interest--Biochemist on staff was approached by NASA-staff prior.
Philips Roxane	-	-	-	-
Richardson-Merrell, Inc.	+	-	10/26/73	Staff will review letter and respond if indicated.
W. H. Rorer, Inc.	+	-	-	-
Schering Corp. Schering Diagnostic	+	-	-	-
Squibb	+	-	-	-
Smith, Kline & French	+	-	-	-
Warner Lambett Pharmaceutical Co.	+	-	-	-
Warren-Teed (Rohm & Haas)	+	-	10/25/73 11/7/73	Sam Gusman has forwarded a copy of the letter to associates at the Rohm and Haas Co. If there is interest they will get in contact
Upjohn Co.	-	+	10/15/73	No interest

*all letters were mailed on 10/17/73

TABLE 8

POPULAR ELECTROPHORESIS ARTICLES

RANK	TIMES CITED	BIBLIOGRAPHICAL DATA
11	3464	Davis, B. J. Disc electrophoresis. 2. Method and application to human serum proteins. Ann. New York Acad. Sci. 121:404-27, 1964.
13	2903	Scheidegger, J. J. Une micro-methode de l'immuno-electrophorese. Internat. Arch. Allergy 7: 103-10, 1955.
26	1841	Smithies, O. Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. Biochem. J. 61:629-41, 1955.
42	1297	Ornstein, L. Disc electrophoresis. 1. Background and theory. Ann. New York Acad. Sci. 121:321-49, 1964.
51	1204	Smithies, O. An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. Biochem. J. 71:585-57, 1959.
63	1066	Poulik, M. D. Starch gel electrophoresis in a discontinuous system of buffers. Nature 180: 1477-79, 1957.
69	1023	Shapiro, A. L., Vinuela, E. and Maizel, J.V., Jr. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Comm. 28:815-20, 1967.

A similar poll of instrument usage by American Laboratory readers shows that nineteen percent of the 2,000 respondents in this survey used electrophoresis equipment. This figure has remained constant over the years around twenty percent usage. The significant fact here is that only eight percent expected electrophoresis to make the greatest contribution in the respondent's field in the next three years. Other facts coming out are that university chemists use electrophoresis equipment significantly more than industrial chemists, and the same is true for university biologists.

Thus, we decided to contact research scientists at leading universities. A Medlar search from the National Library of Medicine was started covering various areas of cell, macromolecular separation, clinical tests, etc. Appendix 3 gives the series of references, organized according to the type of laboratory work they represent (clinical, special techniques, etc.). We selected specific references after all abstracts were scanned for possible interest. An alphabetical listing of the biological materials and special techniques included is given in Appendix 4.

Once experiments which could have potential space remifications were detected we contacted individual scientists who had written the specific articles. The reply letters are given in Appendix 5. An alternate approach was taken by discussing various types of experiments with scientists we met at scientific meetings, including a conference on mammalian cell surfaces (held at Los Alamos Scientific Laboratory) and a National Cancer Institute contractors conference. Most of the responses were either negative or lacked substance. Some of the reluctance on the part of scientists to consider this type of experimentation may be caused by unfamiliarity with the field. If the experiments proposed by NASA on T and B lymphocytes and lipoproteins can be demonstrated to work in space, we would expect many scientists will feel more confident with this program.

A very positive output was obtained from Dr. Leslie Glick, President of Associate Biological Systems, a company which produces large quantities of human cells in culture for specific genetic purposes. Dr. Glick suggested two types of experiments (mentioned in the experiments section): (a) utilization of space cell culture to test the immortality of normal cell lines in the absence of contact inhibition, and (b) utilize the zero g capabilities for attaching cells to floating spheres thus providing a large attachment area; cells which produce vaccines could generate extremely large amounts of material this way as it is not feasible to grow them in monolayers in large areas on earth. Dr. Glick is interested in collaborating with NASA and he sees very positive potential economic applications for the program.

We also evaluated the potential use of space procedures in clinical testing. The most common clinical tests were reviewed (Appendix 6) and some of the needs, which may or may not be met by space, are given in the experiments section. Appendix 6 gives a list of government classified viruses that need further purification.

V. COMPETING TECHNIQUES ON EARTH

In order to consider the possible technical advantage of processing in space, it is also necessary to consider the competing technology and instrumentation on earth. We only include the most relevant procedures, excluding detailed advances in electrophoresis such as precast gels or isoelectric focusing techniques, which we have documented through the extensive bibliography given at the end of the report.

A. MICROSCOPE CELL ELECTROPHORESIS

The apparatus designed by Mehrishi (British Patent No. 2782/71) is extremely inexpensive and can separate populations of cells with computer printout and mobility distributions in short experiments running 10-20 minutes. In this system a very narrow tube is utilized (2 - 2.4 mm) so that no problems of heat convection appear. Mobilities are measured by means of a microscopic system which is focused a distance .293 R from the inner radium of the wall of a tube of radium R. At this point the electro-osmitic effect is zero and the true mobility of the cells is determined. The maximum sensitivity of the system is 5×10^5 to 10^6 ionic groups, mobility changes cannot be determined in heterogeneous cell populations. A change in 10% mobility for a human erythrocyte, for example, (mobility 1.08 microns/sec/V/cm) corresponds to the removal of only 6×10^{-16} gms of NANA (N-acetyl neuraminic acid, responsible for the charges on the surface) from the erythrocyte surface. Although this method is not subject to convection and electro-osmosis, it has the disadvantage, compared to space electrophoresis, that it can only deal with small samples in short times. The space electrophoresis system has the advantage of the long-time capabilities which allow for better separation with the same field strength.

B. FORCED FLOW ELECTROPHORESIS AND ELECTRODECANTATION

These two techniques are aimed at obtaining large fractions of materials with little generation of heat. In electrodecantation, which has been used to prepare immunoglobulin fractions from hyperimmunized plasma, macromolecules are separated on the basis of their size (through the introduction of two semipermeable membranes between the electrodes), sedimentation and mobility properties--as shown in Figure 1.

Forced flow electrophoresis seems to have great potential for fractionation of proteins on a large scale. In this process both electrodecantation and bulk flow is utilized for the fractionation, as shown in Figure 3. The system has been applied to whole plasma for the removal of IgG antibody and it has been automated with controls for temperature, pH, etc., to run for long periods handling volumes of protein solution of 1,000 liters per week.

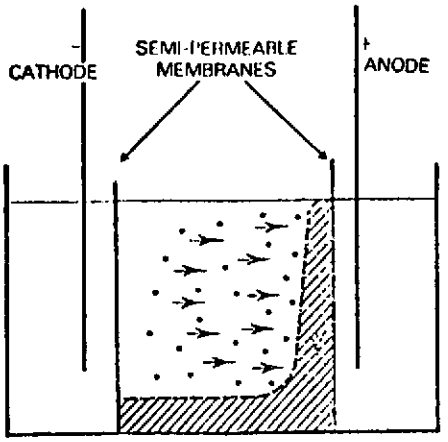

FIGURE 1. ELECTRODECANTATION

HYDROSTATIC GRADIENT

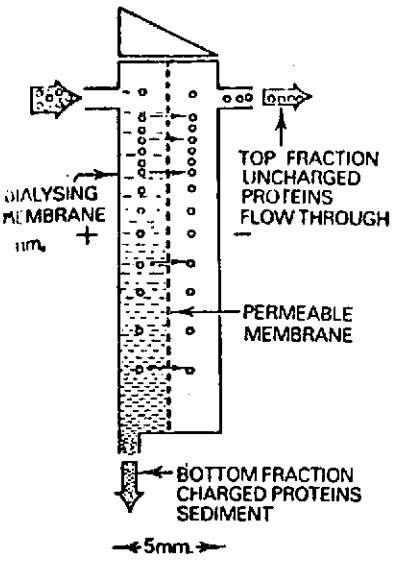

FIGURE 2. FORCED FLOW ELECTROPHORESIS

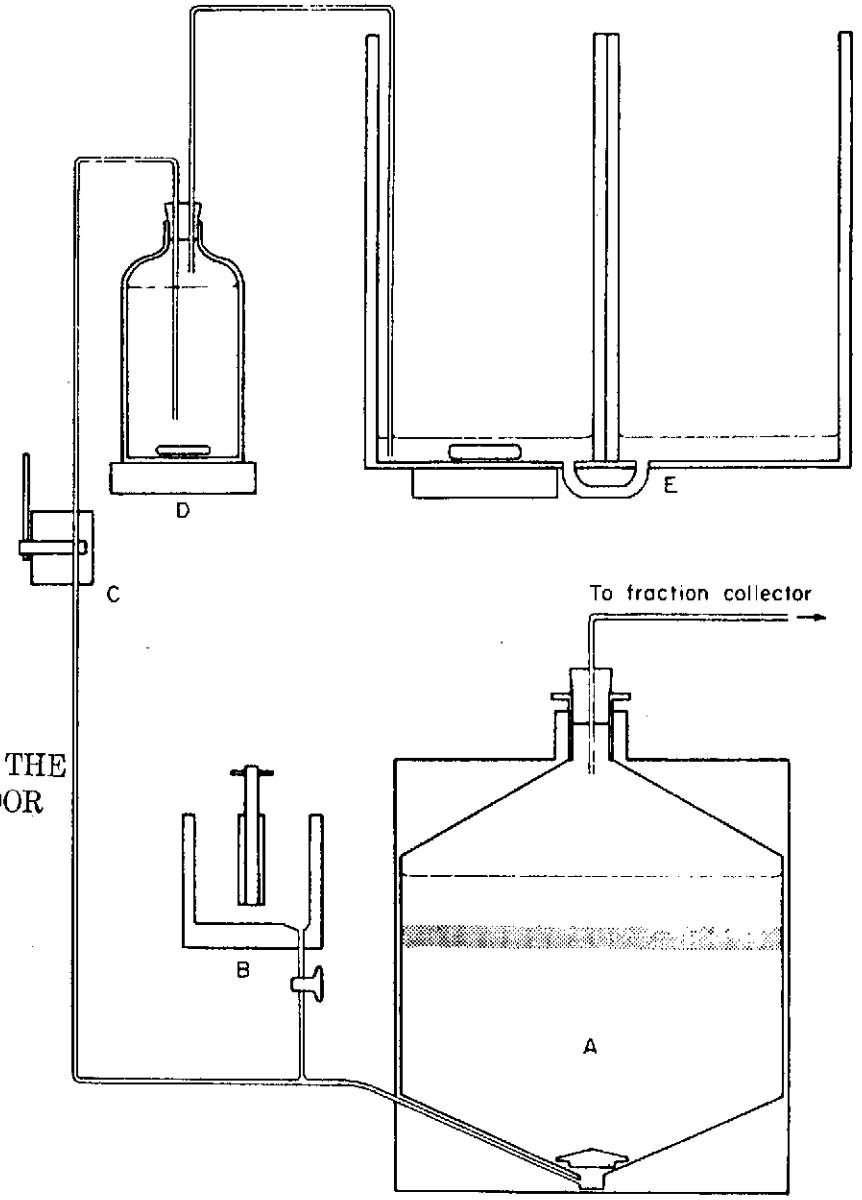

FIGURE 3. APPARATUS FOR 1g SEDIMENTATION OF CELLS

C. DYNAMIC SEDIMENTATION AT 1g

In this system, Figure 4, cells are forced to layer into a narrow band at the same time that a sucrose or other gradient is set in the vessel. After one to two hours of sedimentation, the cells are extracted by forcing them to coalesce into fractions through a very clever inverted funnel system. Cells never reach their equilibrium sedimentation point so that this constitutes a dynamic system. This device is presently utilized to attempt isolation of cells in the G_o stage of the cycle. Although the gradients utilized do not allow separation of T and B lymphocytes at present, it can obtain extremely pure T and B fractions when utilized in conjunction with a Ficoll gradient (personal communication, Dr. R. Barr, National Cancer Institute).

D. CELL SEPARATION BY SELECTIVE ATTACHMENT TO SPECIFIC MATERIALS

Very effective concentrations of viable granulocytes can be obtained by the leukopack, which is a simple sausage containing nylon fibers through which whole blood is passed for transfusion purposes. The granulocytes attach to the nylon fibers in high concentration after several passages of the donor's blood through the system and cells can then be simply extracted with saline. These cells are used for transfusions to leukemic patients.

E. LOS ALAMOS CELL SORTER

A very promising instrument which may find many applications in the coming years is the LASL sorter. This is an instrument which separates single cells into three fractions according to their fluorescence. The fluorescence excitation system is an Argon laser and cells are carried through the air in a drop of saline which gets charged on the basis of the amount of fluorescence in the cell, then deflected by a system similar to a T.V. deflection system. Although the system can now sort cells into only three fractions, it can easily be adapted to classify cells in as many as two fractions on the basis of both size and fluorescence. Large numbers and concentrations of cells can be separated as this instrument handles several thousand cells per minute.

VI. POTENTIAL BIOCHEMICAL AND CELLULAR SPACE PROCESSING AND EXPERIMENTS

A. PROCESSING

PRODUCE LARGE AMOUNTS OF VIRAL VACCINES

DESCRIPTION: CELLS WHICH GROW IN MONOLAYERS IN CULTURE ARE HARD TO OBTAIN IN LARGE QUANTITIES ON EARTH. BY FLOATING SMALL MICROSPHERES IN THE ABSENCE OF GRAVITY VERY LARGE SURFACE TO VOLUME COULD BE OBTAINED AND CELLS WOULD GROW IN MONOLAYERS IN LARGE QUANTITIES, THUS PRODUCING LARGE AMOUNTS OF THE VACCINE. ELECTROENDOSMOSIS COULD INCREASE THE TRANSPORT OF NUTRIENTS AND WASTES THROUGH THE CELL MASS.

PURPOSE: PRODUCE LARGE AMOUNTS OF VIRAL VACCINES BY CELLS WHICH GROW IN MONOLAYERS.

BACKGROUND: EXPERIMENT PROPOSED BY DR. LESLEY GLICK, PRESIDENT OF ASSOCIATE BIOMEDIC SYSTEMS.

PRODUCTION OF EFFECTIVE CANCER ANTIBODIES

PURPOSE: ISOLATE CELLS CAPABLE OF ELICITING EFFECTIVE ANTIBODY PRODUCTION.

PROCEDURE: SEPARATE TRYPSINIZED TUMOR CELLS BY ELECTROPHORESIS. PRODUCE ANTIBODIES USING CELLS FROM VARIOUS FRACTIONS, THEN CHECK FOR MOST EFFECTIVE FRACTION (THE ANTIBODY WILL BE HIGHLY CONCENTRATED IN THIS ONE).

ADVANTAGE: BETTER CELL FRACTIONATION IN SPACE.

POLLEN ELECTROPHORESIS

PURPOSE: ISOLATE POLLENS, TO OBTAIN MORE PURIFIED ALLERGEN FOR BOTH DIAGNOSIS AND TREATMENT.

ADVANTAGES OF SPACE: NO PRECIPITATION, SENSITIVE SEPARATION OF SLIGHT CHARGE DIFFERENCES AND MASS.

PURIFICATION OF ANTISERA

PURPOSE: PURIFY SPECIFIC ANTISERA TO PERFORM RAPID TESTS FOR A2 AND FETAL HEMOGLOBINS, ALKALOIDS, AND LIPOPROTEINS.

DISCONTINUE: NO ADVANTAGE OF SPACE PURIFICATION FOR MACROMOLECULES.

ISOLATE CHROMOSOME FRACTIONS

PURPOSE: BEYOND THE RESEARCH INTEREST THE AVAILABILITY OF SPECIFIC PURIFIED HUMAN CHROMOSOMES WOULD ALLOW DEVELOPMENT OF SPECIFIC FLUORESCENT ANTIBODIES TO THE CHROMOSOMES, SO THAT A RAPID METHOD WOULD BE AVAILABLE FOR DOING CHROMOSOME ANALYSIS ON EARTH.

ADVANTAGE OF SPACE: CHROMOSOME SEPARATION TECHNIQUES ON EARTH DO NOT GIVE PURE CHROMOSOME FRACTIONS, THERE IS CLUMPING AND PRECIPITATION; SPACE ELECTROPHORESIS PURIFICATION WOULD AVOID PRECIPITATION.

PURIFICATION OF ANTIBODIES

PURPOSE: IMPROVE PRESENT CLINICAL TESTS FOR ALDOSTERONE, α-1 FETAL PROTEIN (CANCER OF THE LIVER), RHUMATOID ARTHRITIS (NOW PERFORMED BY LATEX FIXATION TEST).

DISCONTINUE: PURIFICATION OF MACROMOLECULES IN SPACE DOES NOT APPEAR PROMISING.

PURIFICATION OF B- HYDROXYBUTYRATE DEHYDROGENASE

PURPOSE: OBTAIN FOR MORE PRECISE DETERMINATION OF THE IMPORTANT KETONES, AS PRESENT TESTS ARE MORE SEMIQUANTITATIVE THAN PRECISE.

DISCONTINUE: SPACE PROCESSING NOT ADVANTAGEOUS FOR MACROMOLECULAR PURIFICATION.

BONE MARROW CELLULAR SEPARATION FOR TRANSFUSION PURPOSES

DISCONTINUE: EARTH LEUKOPACK® EXTRACTION BETTER AND SIMPLIER FOR MOST TRANSFUSION PURPOSES. NOT ENOUGH VOLUME POSSIBLE IN SPACE. SEPARATION OF BONE MARROW STEM (PRIMORDIAL) CELLS IN SPACE THUS OF ONLY ACADEMIC INTEREST.

B. EXPERIMENTS

TEST PROGRAMMED CELL DEATH THEORY VS. IMMORTAL CELL LINE

PURPOSE: IT HAS BEEN CLAIMED THAT NORMAL CELLS DIFFER FROM CANCER CELLS IN THAT NORMAL CELLS LIVE FOR A PREESTABLISHED NUMBER OF GENERATIONS (HAYFLICK) WHEREAS CANCER CELLS ARE IMMORTAL AND KEEP ON DIVIDING FOREVER. THESE EXPERIMENTS HAVE BEEN PERFORMED IN TISSUE CULTURE ON EARTH WHERE CELLS TOUCH EACH OTHER AND THE GLASS CONTAINER OR PETRIDISH. THUS, IT IS NOT CLEAR WHETHER THERE IS A PREDETERMINED RULE ON PROGRAMMED CELL DEATH OR WHETHER CONTACT INHIBITION AND THE RATE AT WHICH NUTRIENTS CAN BE PROVIDED LIMIT THE NUMBER OF DIVISIONS. IN A GRAVITY SYSTEM IN WHICH CELLS ARE IN SUSPENSION IT WOULD BE POSSIBLE TO TEST THIS THEORY.

BACKGROUND: DR. L. GLICK, PRESIDENT OF ASSOCIATE BIOMEDIC SYSTEMS, HAS PROPOSED THIS EXPERIMENT.

PURIFICATION AND PROPERTIES OF MULTIPLE FORMS OF BRAIN ACETHYCHOLINESTERASE

DESCRIPTION: BRAIN TISSUE CAN BE HOMOGENIZED, THEN PASSED THROUGH FILTERS OF VARYING SIZES TO OBTAIN HOMOGENEOUS MEMBRANE FRACTIONS. THE MEMBRANES COULD BE SEPARATED BY ELECTROPHORESIS IN SPACE AND AFTER ASSAYING FRACTIONS CONTAINING ACETHY-CHOLINESTERASE (OR OTHER INTERESTING MATERIAL) CORRELATED WITH STRUCTURE AND FUNCTION OF THE TISSUE.

PURPOSE: OBTAIN NERVE MEMBRANE FRACTIONS CONTAINING ACETHLCHOLIN-ESTERASE (OR OTHER MARKERS OR SUBSTANCES OF INTEREST).

ADVANTAGES OF SPACE: WITH PRESENT TECHNIQUES MEMBRANES HAVE TO BE SOLUBILIZED AND ARTIFACTS ARE INTRODUCED, EXPERIMENT DISCUSSED WITH DR. JOHN T. DULANEY, DEPARTMENT OF MOLECULAR BIOLOGY, VANDERBILT UNIVERSITY.

STUDY MOBILITIES OF VARIOUS ARTIFICIAL CELL MOSAICS

DESCRIPTION: VARIOUS COMPOUNDS TO WHICH CELLULAR CHARGES ARE USUALLY ASCRIBED (SUCH AS NANA, -SH GROUPS, ETC.) ARE ABSORBED ONTO QUARTZ PARTICLES AND THE RESULTANT ARTIFICIAL CELLS SUBJECTED TO ELECTROPHORESIS.

PURPOSE: DETERMINE EFFECTS OF VARIOUS CHARGED GROUPS ON OVERALL MOBILITY. ALTHOUGH KNOWLEDGE OF THE CELL SURFACE ARCHITECTURE IS EXTREMELY IMPORTANT AND MEASUREMENT OF CELL MOBILITIES ON EARTH IS UTILIZED TO INFER THE TYPES OF CHARGES PRESENT ON THE CELL SURFACE, THERE IS NO RELIABLE WAY OF CONVERTING MOBILITIES INTO COMPOUNDS, AS ALL METHODS ARE INDIRECT (ENZYMATIC TREATMENT OF THE SURFACE) AND NO GOOD MODELS ARE AVAILABLE. SPACE ELECTROPHORESIS OF MODEL SYSTEMS WOULD PROVIDE USEFUL INFORMATION OF THIS TYPE: MODEL CELLS CAN BE ELECTROPHORESED FOR LONG PERIODS OF TIME.

MEASUREMENT: LABEL DIFFERENT "CELLS" WITH VARIOUS STAINS, OR FLUORESCENT LABELS, TAKE PICTURES, NOT NECESSARY TO RETURN SYSTEM TO EARTH. NO PROBLEMS OF KEEPING LIVE BIOLOGICAL MATERIAL, BUT INFORMATION EXTREMELY VALUABLE.

BACKGROUND: SIMILAR EXPERIMENTS DONE WITH LONG CHAIN FATTY ACIDS, ALCOHOLS, AMINES, ETC. BY KRUYT AND WENT, 1931; GROWNEY, 1941; BANGHAM ET AL. 1958. TYPICAL MOSAIC DATA INFERED FOR CELLS GIVEN IN TABLES 9, 10, AND 11 (MEHRISHI, PROGRESS IN BIOPHYSICS AND MOLECULAR BIOLOGY, 1973)

PROBLEMS: NONE FORESEEN, DEFINITE ADVANTAGES OVER EARTH.

TABLE 9

CELL SURFACE MOLECULAR MOSAIC

GROUPS/CHARGES ON THE SURFACE OF HUMAN THROMBOCYTES

Groups or charges	Number per cell ($\times 10^5$)	Area per group/charge ($Å^2 \times 10^3$)	Average distance between neighbouring groups (Å)
1. Positively charged AMINO	2.42	11.7	108
2. Phosphate (alkaline phosphatase-susceptible)	5.0[b]	5.7	75
3. —SH groups	2.75	10.3	32
4. α-Carboxyl (neuraminidase-susceptible-*N*-acetylneuraminic acid)	9.5 8.9	2.98 3.2	55 56
5. Unidentified anionogenic	6.53		
6. Total electron charges	20.42		
7. Net electron	18.0		

TABLE 10

CELL SURFACE MOLECULAR MOSAIC

GROUPS/CHARGES ON THE SURFACE OF HUMAN LYMPHOCYTE

Groups or charges	Number per Cell ($\times 10^5$)	Area per group/charge (Å^2)	Average distance between neighbouring groups (Å)
1. Total electron charges	102.9		
2. Electron charges contributing to the electrokinetic charge	93.4		
3. α-Carboxyl (neuraminidase-susceptible-*N*-acetyl-neuraminic acid)	29.2	3880	62
4. Phosphate (RNase-susceptible)	8.7	13000	114
5. Positively charged amino	9.5	11900	109
6. Unidentified anionogenic	55.5	2050	45
7. —SH groups	19.8	5760	76

TABLE 11

CELL SURFACE MOLECULAR MOSAIC

GROUPS/CHARGES ON THE SURFACE OF EHRLICH ASCITES TUMOUR CELLS

Groups or charges	Number per cell ($\times 10^7$)	Area per group/charge (Å²)	Average distance between neighbouring groups (Å)
1. Total electron charges	8		
2. Electron charges contributing to the electrokinetic charge	6.88		
3. α-Carboxyl (neuraminidase-susceptible-*N*-acetyl-neuraminic acid)	2.35	3830	61.9
4. Phosphate (RNase-susceptible)	1.19	7550	86.9
5. Positively charged amino	1.17	7700	87.6
6. Unidentified anionogenic	3.91	2300	48
7. —SH groups	3.69	2440	49.4

EFFECTS OF CHEMOTHERAPEUTIC AGENTS ON MOBILITIES OF NORMAL AND CANCER CELLS

DESCRIPTION: CHEMOTHERAPEUTIC AGENTS ADDED IN SMALL DOSAGES TO BOTH NORMAL AND CANCER CELLS OF VARIOUS TYPES TO STUDY SLIGHT CHANGES IN MOBILITY AND MOBILITY VARIATIONS WITH INCREMENTAL CHANGES IN DOSAGE.

PURPOSE: IT IS IMPORTANT TO FIND SIMPLE IN VITRO TECHNIQUES FOR SCREENING CHEMOTHERAPEUTIC AGENTS.

ADVANTAGE OF SPACE: VERY SMALL CHANGES IN MOBILITY COULD BE OBSERVED. AS THE SCREENING PROCESS IS EXTREMELY EXPENSIVE ON EARTH ANY PROMISING TECHNIQUE FOR REDUCING THE SCREENING TIME SHOULD BE INVESTIGATED AND THE SPACE PROGRAM WOULD BE COMPETITIVE EVEN AT THE COST LEVEL.

CORRELATIONS BETWEEN CHANGES IN SURFACE CHARGE DENSITY AND CELL CONTACT BEHAVIOUR

DESCRIPTION: STUDY CHANGES IN MOBILITIES IN RESPONSE TO CONTACT BEHAVIOUR

DISCONTINUE: LACK OF CORRELATION BETWEEN CHARGE AND CONTACT BEHAVIOUR IN EXPERIMENTS PERFORMED ON DISSOCIATED CHICK EMBRYO CELLS ON EARTH (KEMP, 475; JOHNES, 476)*

* NUMBERS REFER TO THE MEDLINE REFERENCES IN OUR STUDY, SEE APPENDIX 3.

PREORDAINED FUNCTION IN EMBRYOGENESIS

DESCRIPTION: DEAGGREGATE CELLS IN A GIVEN EARLY EMBRYONIC STAGE AND MEASURE RESULTANT MOBILITIES.

PURPOSE: STUDY MOBILITIES OF CELLS AT VARIOUS STAGES OF EMBRYOGENESIS TO DETERMINE, THROUGH THESE DIFFERENCES, IF CELLS WHICH LOOK MORPHOLOGICALLY THE SAME MAY HAVE A PREORDAINED FUNCTION, AS JUDGED THROUGH DIFFERENCES IN MOBILITY.

BACKGROUND: STUDIES ON PRESUMPTIVE EPIDERMIS OF THE FROG SHOWS THAT CELLS WHICH ARE DEVELOPMENT ALLY AHEAD OF OTHERS SHOW DIFFERENT ELECTROPHORETIC MOBILITY. DASQUPTA, KUNG HO (336).

ADVANTAGE OF SPACE: IN ADDITION TO THE USUAL CELL ELECTROPHORESIS PROBLEMS, IT WOULD BE POSSIBLE TO DETECT SMALLER DIFFERENCES IN MOBILITIES, HENCE DETECT PREORDAINED FUNCTION AT EARLIER STAGES (THE TWO CELL LEVEL) IF IT EXISTS.

SEPARATION OF BONE MARROW CELLS TO STUDY HEMOPOIESIS

DESCRIPTION: MORPHOLOGICALLY SIMILAR BONE MARROW CELLS ARE SEPARATED BY ELECTROPHORESIS AND CULTURED. CULTURES RECOVERED AND STUDIED TO OBTAIN PURE GRANULOCYTE CELL LINES.

PURPOSE: EXTREMELY IMPORTANT IN THE DETERMINATION OF THE CAUSE OF LEUKEMIA TO OBTAIN PURE GRANULOCYTE LINES, THIS HAS NOT BEEN ACHIEVED BY ANY TECHNIQUE SO FAR.

ADVANTAGES OF SPACE: DETECTION OF VERY SMALL CHARGE DIFFERENCES IN CELLS WHICH ARE MORPHOLOGICALLY AND FUNCTIONALLY VERY CLOSE TO EACH OTHER.

STUDY MARKERS OF TUMOR GROWTH

DESCRIPTION: SUSPEND CELLS AT THEIR ISOELECTRIC POINT AND ALLOW SMALL MOLECULES TO MOVE IN THE ELECTROPHORETIC FIELD. AFTER A FEW HOURS, EXTRACT SAMPLES FROM VARIOUS PARTS OF THE ELECTROPHORESIS TUBE AND SEND SAMPLES OF THE SMALL MOLECULES TO EARTH.

PURPOSE: IT MAY BE USEFUL TO KNOW WHAT SUBSTANCES (MOSTLY SMALL MOLECULES) ARE PRODUCED UNIQUELY BY TUMOR CELLS.

DISCONTINUE: MAMMALIAN CELLS DO NOT SURVIVE AT THEIR ISOELECTRIC POINT.

SEPARATION OF X AND Y OR SPERM CARRYING DIFFERENT GENES

PURPOSE: OBTAIN SPERM CARRYING X OR Y CHROMOSOMES ONLY, OR SPERM CARRYING SPECIFIC GENETIC MUTATION.

DISCONTINUE: BACKGROUND ON EARTH SHOWS THAT ELECTROPHORESIS DOES NOT EFFECT THE SEPARATION. THE EARTH TECHNIQUE TO SEPARATE X AND Y SPERM (DR. R. J. ERICKSON) IS BASED ON DIFFERENTIAL MOTILITY OF SPERM IN TWO MEDIA OF DIFFERENT VISCOSITY: SPERM REQUIRE GRAVITY FOR ORIENTATION.

STUDY PLATELET - MATERIAL INTERACTION

DESCRIPTION: A DISPERSION OF A SAMPLE MATERIAL IS INTRODUCED IN THE ELECTROPHORESIS CELL IS SPACE, THE PARTICLES FLOAT RATHER THAN SEDIMENT. PLATELETS CAN BE RUN THROUGH THE MATERIAL SUSPENSION DRIVEN BY THE ELECTROPHORETIC FIELD AND THE NEGATIVE CHARGE ON THE PLATELET. THE INTERACTION OF THE MATERIAL WITH THE PLATELET WILL DETERMINE MIGRATION RATE.

PURPOSE: STUDY THE BEHAVIOUR OF MATERIALS UTILIZED IN THE ARTIFICIAL HEART PROGRAM, THE NATIONAL HEART AND LUNG INSTITUTE IS CURRENTLY INTERESTED IN ANALYSING INTERACTIONS OF MATERIALS AND BLOOD COMPONENTS.

ADVANTAGES OF SPACE: EQUIVALENT EXPERIMENT IMPOSSIBLE TO PERFORM ON EARTH.

ANALYSIS OF NON-HISTONE NUCLEAR PROTEINS

PURPOSE: ISOLATE PROTEINS INVOLVED IN CONTROL OF GENETIC EXPRESSION. THESE ARE INSOLUBLE COMPLEXES THAT ARE HARD TO ANALYZE AND CANNOT BE SEPARATED ON POLYACRYLANIDE GEL OR BY ISOELECTRIC FOCUSING BECAUSE MATERIALS ARE INSOLUBLE AT THEIR ISOLELECTRIC POINT.

ADVANTAGES OF SPACE: PRECIPITATION WOULD NOT OCCUR.

ISOLATE CELLS IN THE G_o STAGE OF THE CELL CYCLE

DESCRIPTION: CELLS ARE SUBJECTED TO ELECTROPHORESIS AS THEY GO THROUGH MITOSIS IN THE PRESENCE OF VARIOUS DRUGS. THE SPECIFIC CELLS WHICH DO NOT DIVIDE (CHARGE REMAINS CONSTANT) WILL BE RECOVERED.

PURPOSE: TO UNDERSTAND THE EFFECTS OF DRUGS WHICH SEND CELLS TO THE G_o (RESTING) STAGE. THIS HAS IMPORTANCE IN THE TREATMENT OF CANCER.

ADVANTAGES OF SPACE: THERE ARE NO OBVIOUS MORPHOLOGICAL DIFFERENCES BETWEEN CELLS IN G_o AND G_1, HOWEVER, SMALL DIFFERENCES IN CHARGE ARE TO BE EXPECTED.

ISOLATION OF MICROTUBULES OF MITOTIC SPINDLE

PURPOSE: UNDERSTAND STRUCTURE AND FUNCTION OF SPINDLE DURING MITOSIS, SEVERAL GROUPS THROUGHOUT THE UNITED STATES ARE WORKING ON THIS.

PROCEDURE: LYSE CELLS IN VARIOUS STAGES OF MITOSIS, PERFORM ELECTROPHORESIS IN SPACE TO OBTAIN PURE FRACTIONS.

ADVANTAGES: AS IN THE CASE OF CHROMOSOMES, IT IS DIFFICULT TO OBTAIN PURE INSOLUBLE FRACTIONS.

EFFECTS OF SPACE RADIATION ON CELL MOBILITY

DESCRIPTION: A SHIELDED AND AN UNSHIELDED SET-UP IN WHICH CELLS ARE SUBJECTED TO AN ELECTRIC FIELD WILL BE COMPARED FOR CELLULAR MOBILITY.

PURPOSE: TO STUDY POSSIBLE SUBTLE ORBITAL RADIATION EFFECTS.

BACKGROUND: IT IS KNOWN THAT RADIATION HAS EFFECTS ON ELECTROPHORETIC CELL MOBILITIES (MERISHI).

ADVANTAGES OF SPACE: IN THIS PARTICULAR APPLICATION "SPACE" PROVIDES THE RADIATION DOSAGE ALLOWING A CHECK ON THE EFFECTS OF SPACE TRAVEL ON CELLS SUCH AS BLOOD CELLS.

CULTURING OF MAMMALIAN CELLS WHICH WILL NOT GROW IN CULTURE

(NOT INVOLVING ELECTROPHORESIS)

PURPOSE: GROW IN LARGE QUANTITIES CELLS WHICH ARE REQUIRED FOR RESEARCH PURPOSES AND CANNOT GROW IN SUSPENSION ON EARTH AT 1g WHICH DISRUPTS MANY CELLS, AS THE CELLS MUST BE ARTIFICIALLY SUSPENDED BY AGITATION OF THE MEDIUM.

ADVANTAGE: NO SEDIMENTATION IN SPACE.

ISOLATED HUMAN CHROMOSOME FRACTIONS FOR MOLECULAR BIOLOGY STUDIES

PURPOSE: ISOLATE FRACTIONS CONTAINING A FEW GENES, WHICH CAN THEN BE USED FOR CORRELATING STRUCTURE AND FUNCTION.

ADVANTAGES OF SPACE: CHROMOSOME FRACTIONS GIVE INSOLUBLE, TANGLED COMPLEXES UTILIZING CENTRIFUGATION ON EARTH.

OBSERVE MOBILITY CHANGES IN RESPONSE TO ONCOGENIC AND OTHER VIRUSES

PURPOSE: STUDY ELECTROPHORETIC MOBILITY OF CELLS IN RESPONSE TO CANCER AND OTHER DISEASE PRODUCING VIRUSES TO ANALYZE CHANGES IN MEMBRANE STRUCTURE.

BACKGROUND: THE ELECTROPHORETIC MOBILITY OF INTESTINAL EPITHELIAL CELLS WAS MEASURED IN THE PRESENCE AND ABSENCE OF CHOLERA ORGANISMS AND DIFFERENCES IN MOBILITY WERE OBSERVED, EVEN THOUGH STRUCTURALLY THE TWO TYPES OF CELL LOOKED THE SAME. THIS INDICATES THE APPROACH MIGHT SERVE AS A GOOD TEST FOR DETECTING SMALL CELLULAR CHANGES WHICH FOLLOW INFECTION.